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Experimental study of the development of the truncus and the conus in the chick embryo

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INTRODUCTION

There are numerous publications dealing with the normal development of the conus (primordium of the infundibulum) and the truncus (aortic bulb) (Davis, 1927; Kramer, 1942; De Vries & Saunders, 1962; Grant, 1962; Van Mierop, Alley, Kausel & Stranahan, 1963; Van Mierop & Netter, 1969; Asami, 1969; De la Cruz, Muñoz-Armas & Muñoz-Castellanos, 1971; Goor, Dische & Lillehei, 1972; Anderson, Wilkinson & Lubkiewics, 1974). These authors invariably begin with the primitive cardiac cavities, establishing their limits, and go on to describe their structural changes during development and their location in the mature heart, using descriptive embryological techniques. Such techniques, however, are inadequate because of the distortion inevitable in fixed preparations, because structural changes cannot be followed in the same heart and because, some features apparent *in vivo* are not detectable in fixed material. Experimental methods using *in vivo* labelling techniques on the other hand permit precise identification of the fates of the primitive cardiac cavities throughout development.

Previous experimental work (Stalsberg & De Haan, 1969; Castro-Quezada, Nadal-Ginard & De la Cruz, 1972; Argüello, De la Cruz & Sánchez, 1975), done at the stage of first appearance of the primitive cardiac cavities, leads us to suspect that the conus and the truncus are not yet present at the stage of fusion of the epimyocardial troughs; rather that they appear in subsequent stages of development. In order to ascertain the stage of appearance of the conus and the truncus, their evolution during development, and their anatomical manifestations in the mature heart, *in vivo* labelling experiments were carried out on chick hearts from stage 9⁻ to stage 35 of Hamburger & Hamilton's (1951) schedule of development. As well, microdissections and histological and cinematographic studies were carried out and the anatomy of the relevant regions in mature hearts was compared in chick and man.

MATERIAL AND METHODS

In order to locate the prospective conus and truncus regions, *in vivo* labelling experiments were undertaken. Once these regions had been identified, their microscopical anatomy and histology were investigated.

Experiments were carried out on embryos from fertile White Leghorn hens' eggs incubated at 38 °C with 86–87 % humidity. The age of each embryo was determined by reference to Hamburger & Hamilton's (1951) schedule.

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Labelling experiments

Search for the prospective truncus and conus involved labelling different regions of the developing heart at stages 9⁻, 12 and 22 and tracing these labels to stage 35, when the truncus and conus are well defined anatomically. The appearance of new segments of the heart at stages 12 and 22 led to the decision to place new labels, and to divide the experiments into three series.

Because in the early stages of the development of the heart (stage 9⁻) the embryo rests with its ventral surface on the yolk, labelling was done *in vitro*. *In vitro* culture techniques at present do not permit normal development of the heart after stage 12. Therefore, at later stages, labelling was done *in ovo*.

After being labelled, embryos from the different series were reincubated in a hot pack at 37·5 °C, 100% humidity and 5% O_2.

Series 1. (Stage 9⁻.) This experiment was undertaken in order to confirm whether the region of fusion of the two epimyocardial troughs really corresponds to the prospective conus, as has been asserted (Stalsberg & DeHaan, 1969; Castro-Quezada *et al.* 1972) and also to investigate the prospective fate of the region cephalic to those troughs, which is also a cardiogenic area (Argüello *et al.* 1975). Thirty embryos at stage 9⁻ were cultured with their ventral surface turned upward, using New's (1955) technique, and they were dissected by the technique of Castro Quezada *et al.* (1972). Iron oxide particles were placed at three different levels: particle (*a*) in the caudal end of fusion of both myocardial troughs; particle (*b*) in the rostral end of fusion of the same troughs; particle (*c*) in the ventromedial zone of the subcephalic fold (Figs. 1A and 2A). The embryos were cultured until they reached stage 12.

Series 2. (Stage 12.) This experiment was carried out in order to investigate the prospective fate of the new cardiac segment (probably conus) which appeared cephalad to label (*c*) of the previous series (compare Figs. 1A and 2A with Figs. 1B and 2B). For this purpose in a group of 36 embryos, at stage 12, a label was placed in the groove considered by Davis (1927) as 'right interbulbar', the site where label (*c*) was found at the end of the experiment in series 1 (Figs. 1B and 2B, C). In another group of 30 embryos a new label was placed (*d*) in the cephalic end of this new segment (Figs. 1B and 3A). Because labels (*a*) and (*b*) in the previous experiment were found in the prospective area of the trabeculated region of the right ventricle (Stalsberg & DeHaan, 1969; De Vries & Saunders, 1962) they were omitted in this series (Figs. 1A, B and 2A, B). Labelling in this series was done *in ovo* introducing a fine glass filament covered with a gelatin–India ink mixture for 15 minutes (Seichert's technique, 1965). The filament was subsequently drawn out leaving a black mark in the cardiac tissue. In order to place this label, a window, measuring approximately 1 cm^2, was opened in the egg shell; the vitelline and pericardiac membranes were dissected in order to expose the heart. After labelling the window was covered with a transparent Kleen-Pack tissue and the embryos were reincubated until they reached stage 22.

Series 3. (Stage 22.) This experiment was carried out in order to continue tracing the segment of the heart cephalic to label (*c*) (probably the conus) (Figs. 1B and 2B) and to investigate the possible prospective region of the truncus. Thirty embryos at stage 22 were labelled at the boundary between the smooth region (region of the

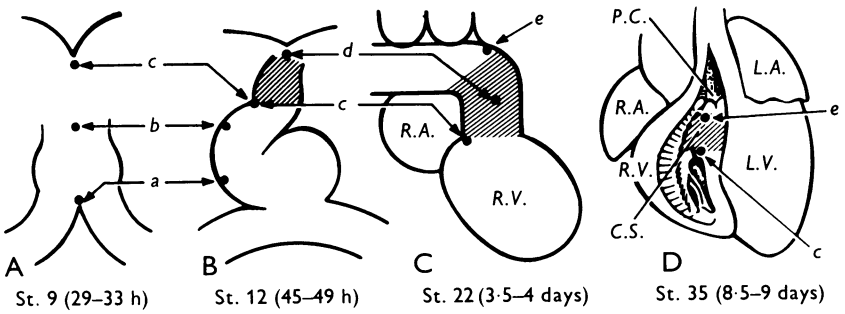


Fig. 1. Diagrammatic representation of results obtained by means of *in vivo* labelling techniques concerning the stage of appearance of the conus, its limits and its relations with the truncus and the ventricles, as well as its anatomical manifestations. Observe the position of label (*c*) caudal to the new segment of the heart in stage 12, and its final location in the lower border of the horizontal portion of the crista supraventricularis (*C.S.*) in stage 35, which indicates that the conus appears at stage 12. The final situation of label (*d*) at stage 22, placed in the cephalic end of the cardiac tube at stage 12, indicates that at this stage there is still no truncus. The final position of label (*e*) beneath the pulmonary semilunar valve cusps (*P.C.*) at stage 35 indicates that the area in which that label was placed in stage 22 corresponds to the boundary between the conus and the truncus. Striped area, conus; *R.A.*, right atrium; *L.A.*, left atrium; *R.V.*, right ventricle; *L.V.*, left ventricle.

primordium of the infundibulum according to De Vries & Saunders, 1962), or conus, and the trabeculated zone of the right ventricle (Figs. 1C and 3C), at the site where the (*c*) label was found at the end of the experiment in series 2 (Figs. 1C and 2D). In addition, a new label was placed (*e*) at the site which has long been described in descriptive embryology (Von Haller, 1758; De Vries & Saunders, 1962) as the probable site of the infundibulo-truncal junction (the boundary between the conus and the truncus) (Figs. 1C and 3C). Taking into consideration these facts, a (*d*) label was not placed in this series (Fig. 3B). Labels were placed *in ovo* in these embryos with the gelatin–India ink technique and they were reincubated until they reached stage 35 (Figs. 1D and 3D). The embryos were fixed in 70° alcohol, and the heart was dissected in search of the labels. The labelled hearts of the embryos in the three series were compared with those of control embryos of the same age.

Anatomical microscopic studies

With the purpose of describing the anatomical features of the conus and the truncus, stage 22, in which both regions of the embryonic heart are evident, was selected, (Figs. 3C and 4B, C). Transverse sections were made at the level of the atrioventricular canal and of the conus in order to investigate the possible presence of two conus chambers and their relations with the ventricles and with the atrioventricular canal (Fig. 4A). Microdissections of the conus and the truncus were also made from their right aspects to further their anatomical study (Figs. 4B, C). In addition cinematography was carried out in order to study the blood flow, and to visualize the conus and the truncus ridges as well as the relations between the conus and the atrioventricular canal and the ventricles, *in vivo*.

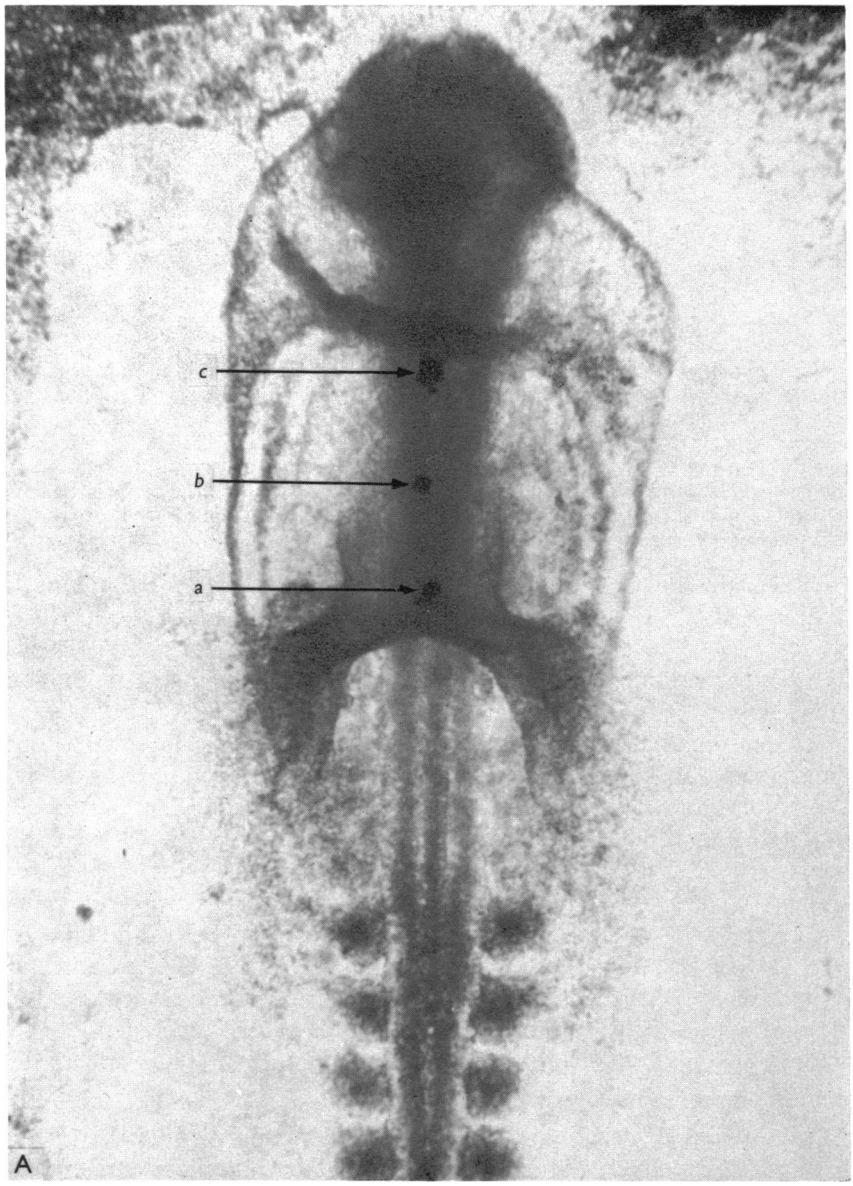
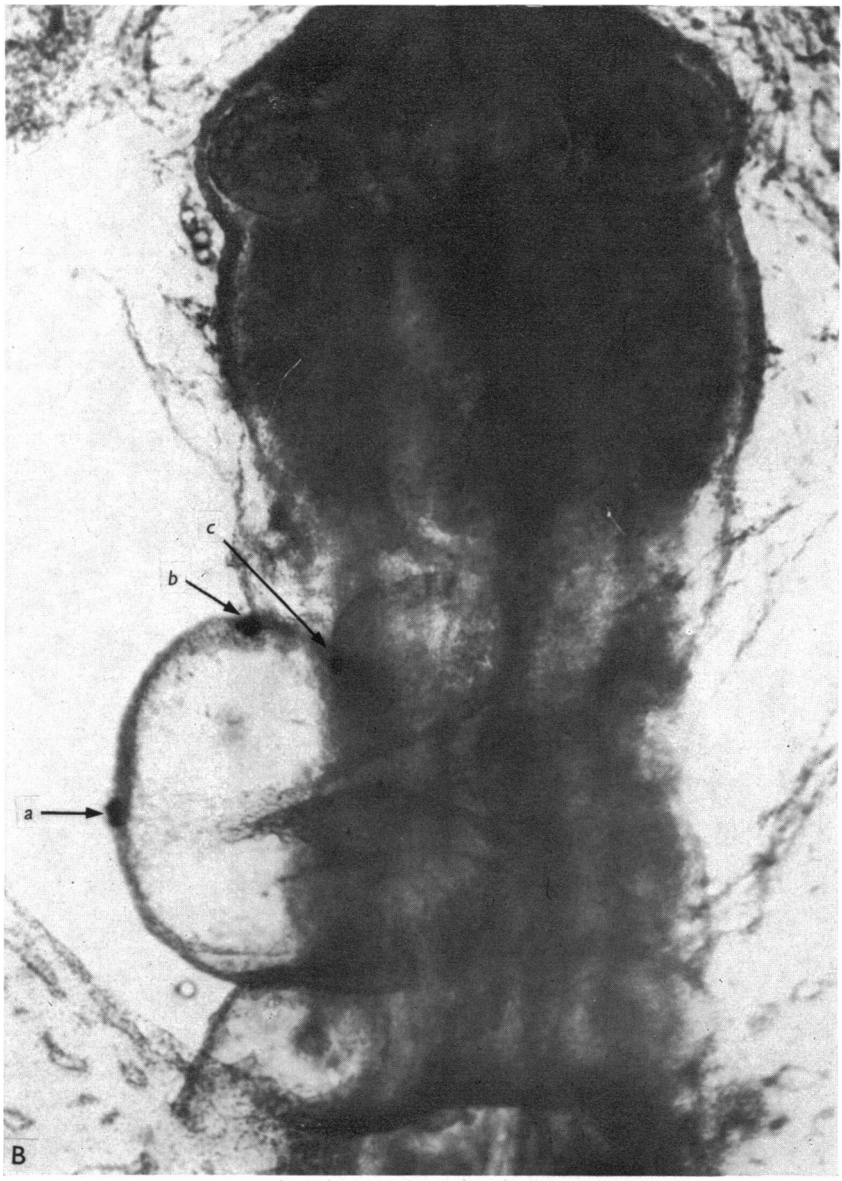


Fig. 2. *In vivo* labelling showing the stage in which the prospective conus appears, and its tracing up to stage 22. (A) Embryo at stage 9⁻ after having placed three particles of iron oxide; (*a*) in the caudal end of fusion of the myocardial troughs; (*b*) in the rostral end of fusion of these troughs and (*c*) in the ventromedial area of the subcephalic fold. × 160. (B) Observe in the same embryo at stage 12 that the particles (*a*) and (*b*) are now located in the primordium of the trabeculated portion of the right ventricle, and that cephalic to particle (*c*) a new segment of the heart has appeared. × 135. (C) Embryo at stage 12 with a gelatin–India ink label placed where particle (*c*) was found in the previous embryo. × 130. (D) Observe the same embryo at stage 22, showing that the ink label (*c*) has appeared in the boundary between the conus area and the trabeculated portion of the right ventricle. × 70.



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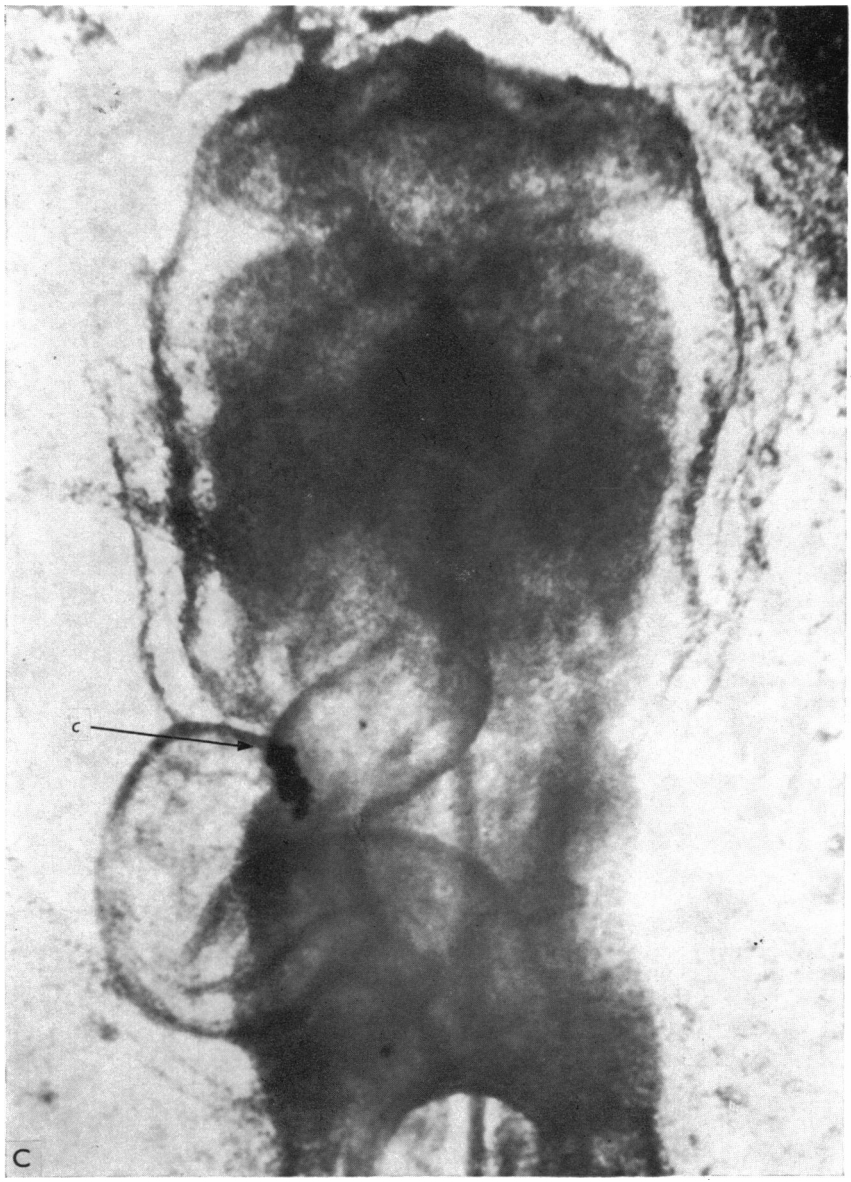
In order to prove that at stage 35 the infundibulum of the right and left ventricles, the interventricular septum, and the valvular floors of the great arteries and their trunk have reached full development, microdissections of these areas were carried out (Figs. 5A, C and 6A, C).

In order to ascertain the degree of similarity between the chick heart at stage 35



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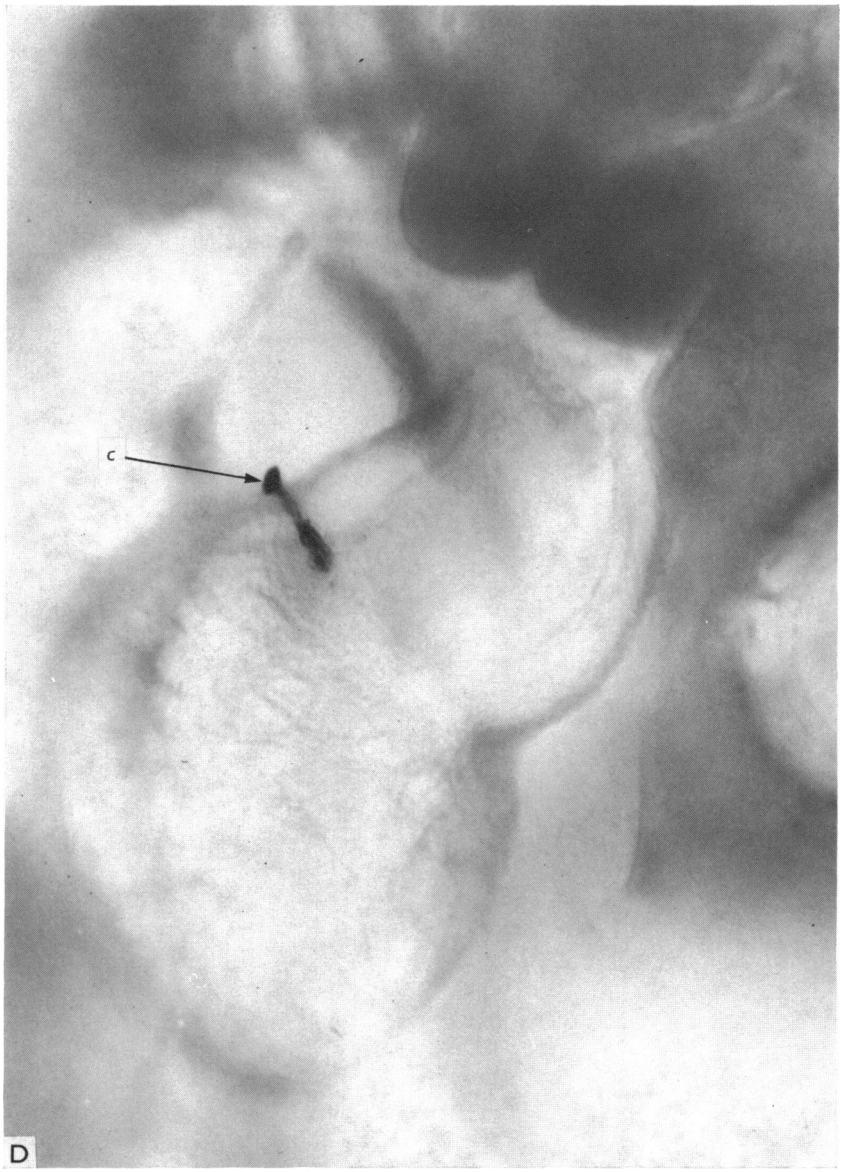
and the mature human heart, a comparative study was undertaken of the anatomica areas mentioned by means of dissections and transverse sections (Figs. 5 and 6).

Histological studies

Histological sections were made of the truncus and conus regions of the heart at stage 22 and compared with the sections of the infundibulum of the right ventricle



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and the trunk of the pulmonary artery at stage 35 (Fig. 7). The tissues were fixed in 2·5% glutaraldehyde in 0·1 M cacodylate buffer, embedded in Epon 812 and 1 μm sections were cut with the Reichter UM3 ultramicrotome. The sections were stained with toluidine blue.

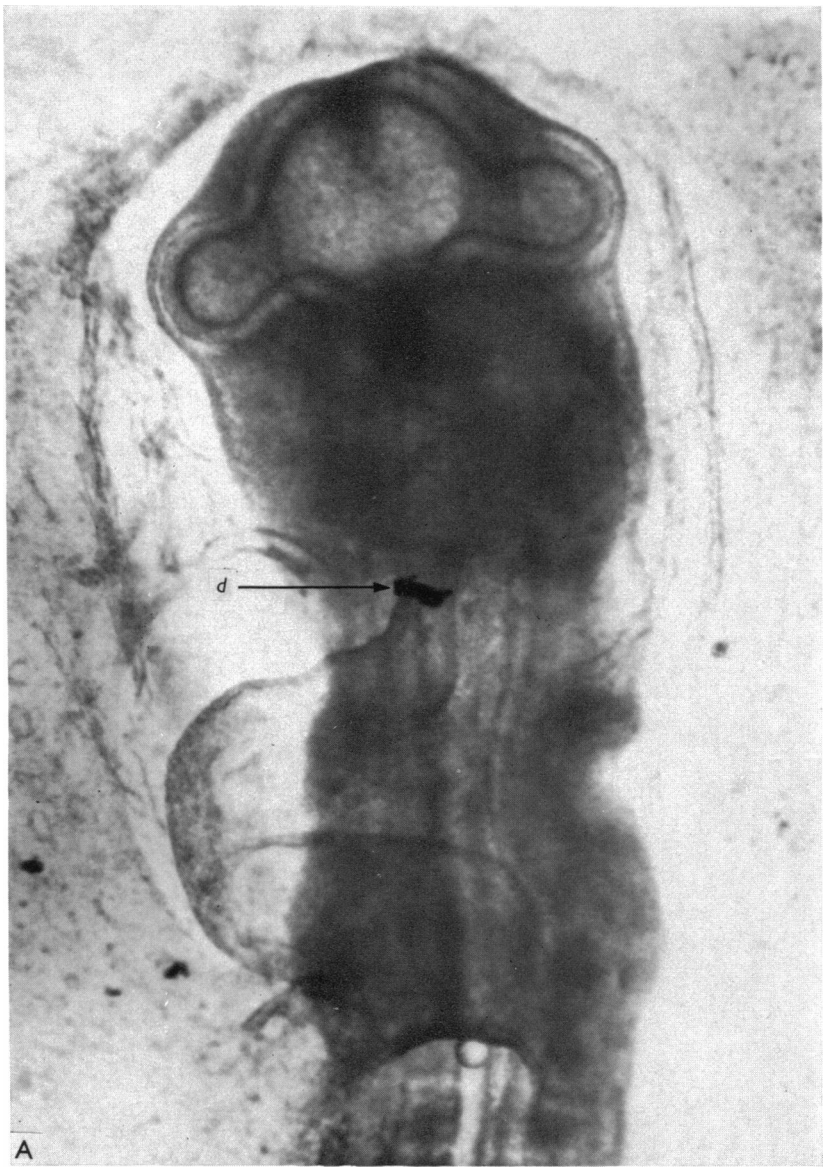
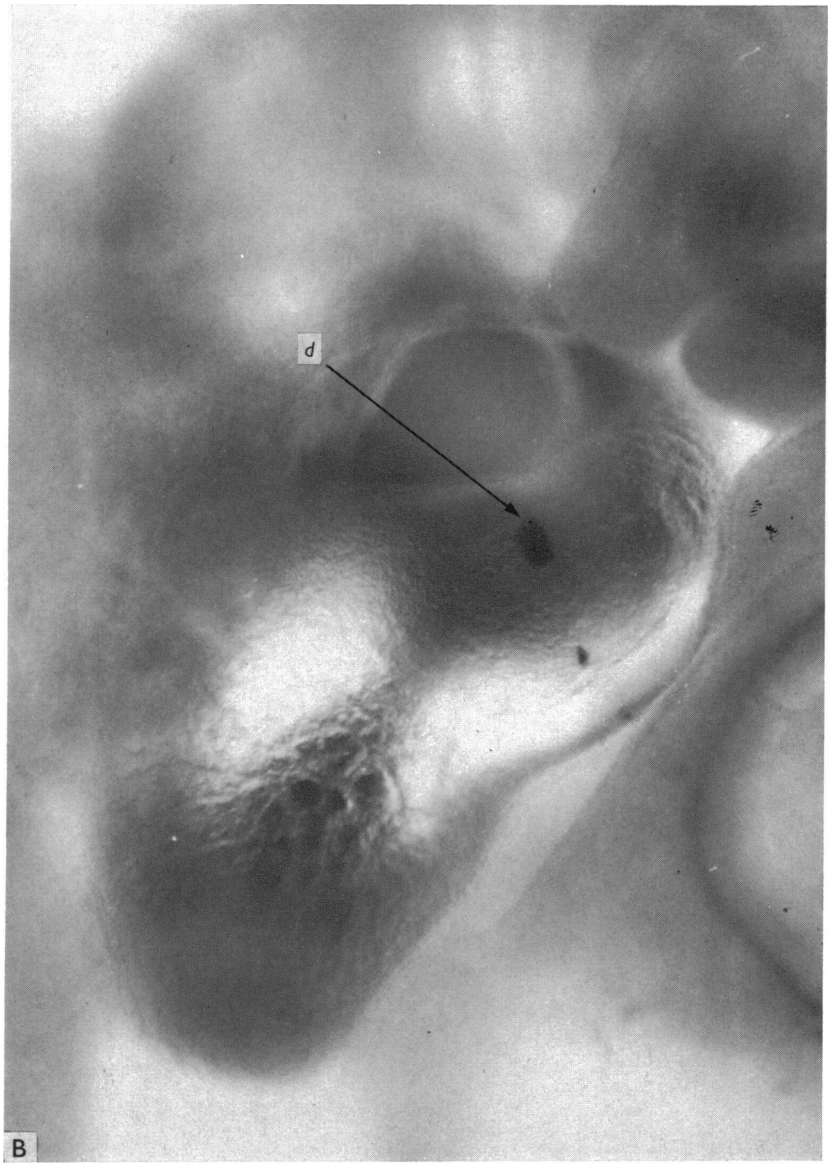


Fig. 3. *In vivo* labelling showing the fate of the conus between stages 12 and 35, its limits, its relations with the truncus, and its anatomical appearance. (A) Embryo at stage 12 with a label (*d*) (gelatin–India ink) placed in the cephalic end of the cardiac tube. × 130. (B) The same embryo at stage 22 showing the label (*d*) in the middle area of the conus region. × 65. (C) Embryo at stage 22 with two labels of gelatin–India ink. Notice that label (*c*) is in the same place in which label (*c*) was found in an embryo of the same age (boundary between the conus and the trabeculated portion of the right ventricle), and also a new label (*e*) in the probable cephalic end of the conus. × 65. (D) The same embryo at stage 35. Observe label (*c*) in the lower edge of the horizontal portion of the crista supraventricularis (*C.S.*) in the infundibulum of the right ventricle (*R.V.*), and label (*e*) beneath the annulus of the semilunar pulmonary valve cusps (*P.C.*). This experiment shows that the caudal end of the embryonic conus corresponds in the right ventricle to the lower edge of the horizontal portion of the crista supraventricularis and its upper limit is located under the semilunar pulmonary valve cusps. *R.A.*, right atrium; *L.A.*, left atrium; *L.V.* left ventricle; *P.T.*, pulmonary trunk. × 27.



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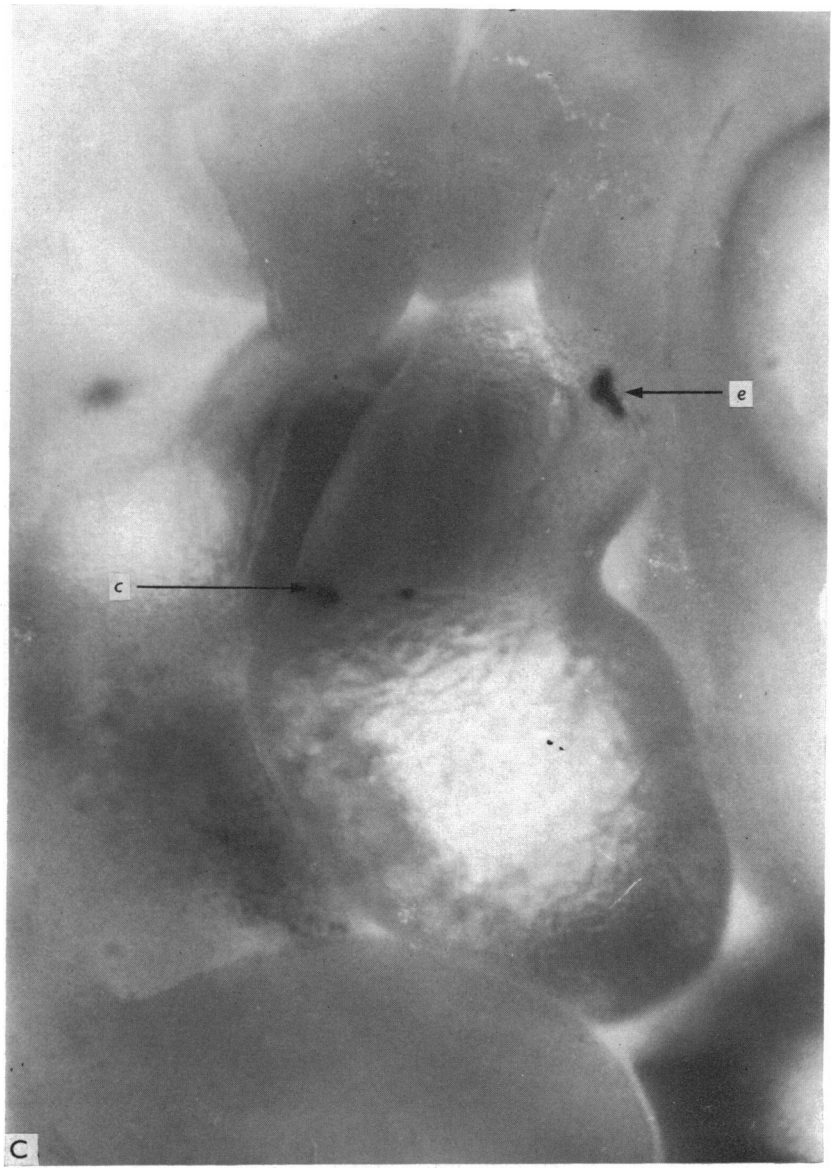
RESULTS

Labelling experiments

Series 1. The thirty embryos labelled at stage 9⁻ exhibited the labels in stage 12 with the following distribution. Label (*a*) was found in the convexity of the bulboventricular loop opposite the left interventricular groove. Label (*b*) was found in the



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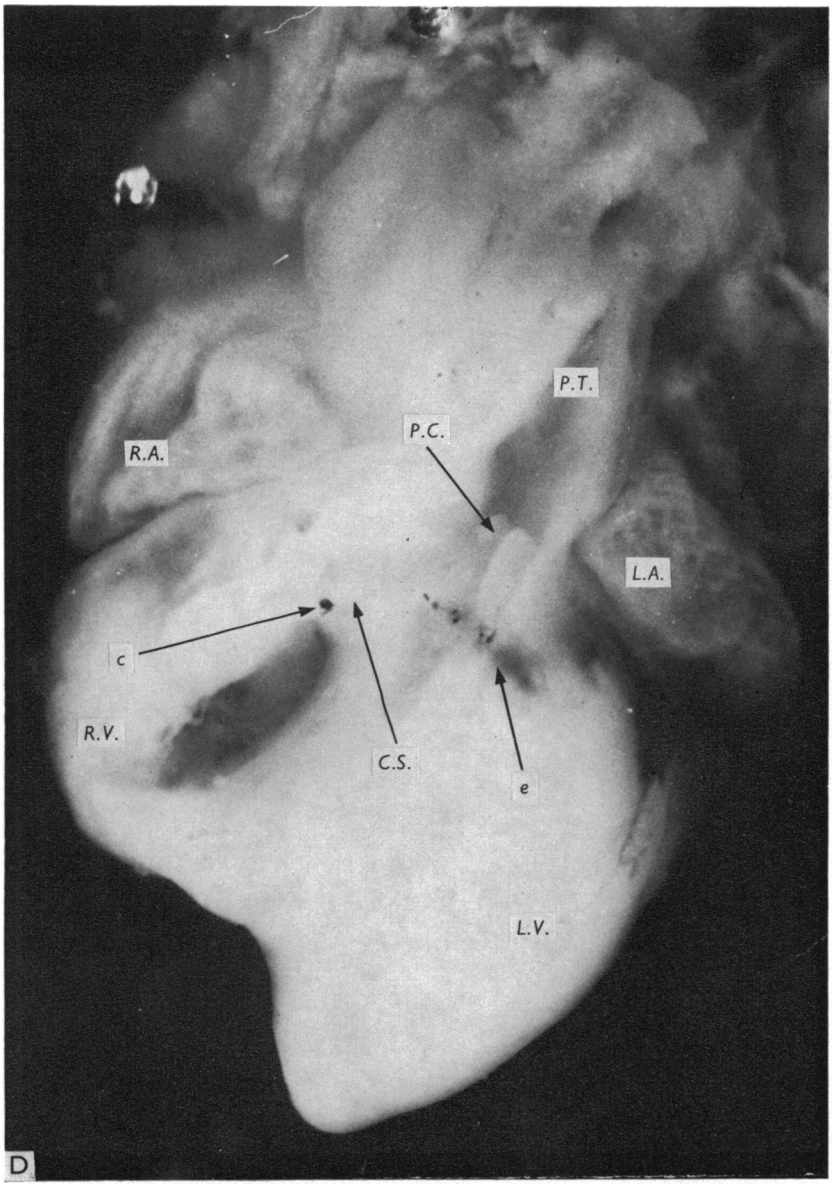
primordium of the trabeculated region of the right ventricle. Label (*c*), which at stage 9⁻ was in the ventromedial zone of the subcephalic fold, was found in the groove that Davis (1927) called the right interbulbar. Rostral to this label a new segment of the heart appeared, as was demonstrated by Argüello *et al.* (1975) (compare Figs. 1A and 2A with Figs. 1B and 2B).

Series 2. Of the 36 embryos labelled at stage 12 in the groove which Davis (1927)



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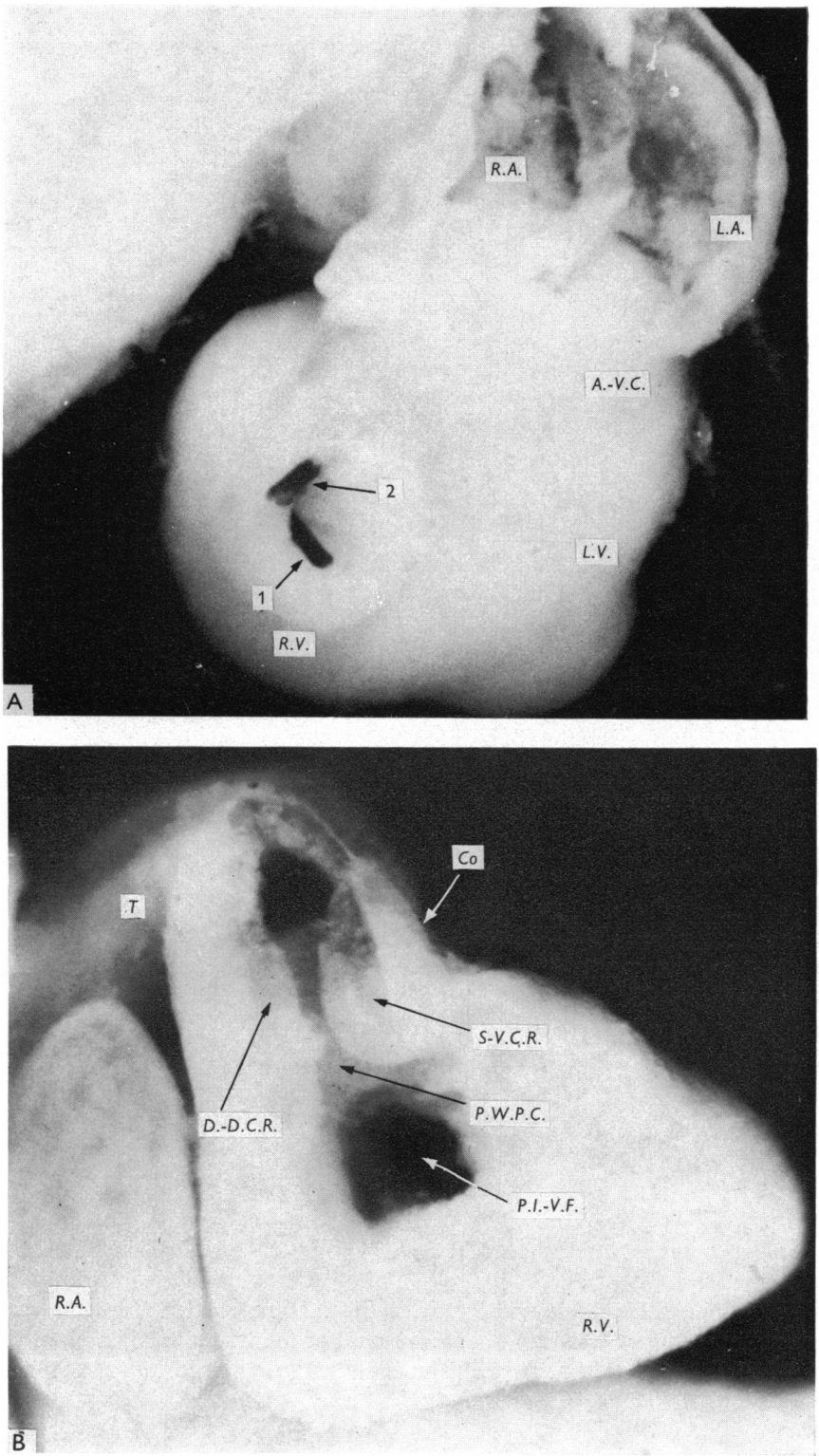
considered as the right interbulbar (label *c*), 34 embryos exhibited the label at stage 22 at the boundary between the conus region and the trabeculated portion of the right ventricle (compare Figs. 1B and 2C with Figs. 1C and 2D). The two remaining embryos exhibited this label in the anterior wall of the trabeculated area of the right ventricle. The 30 embryos at stage 12 labelled at the cephalic end of the heart tube



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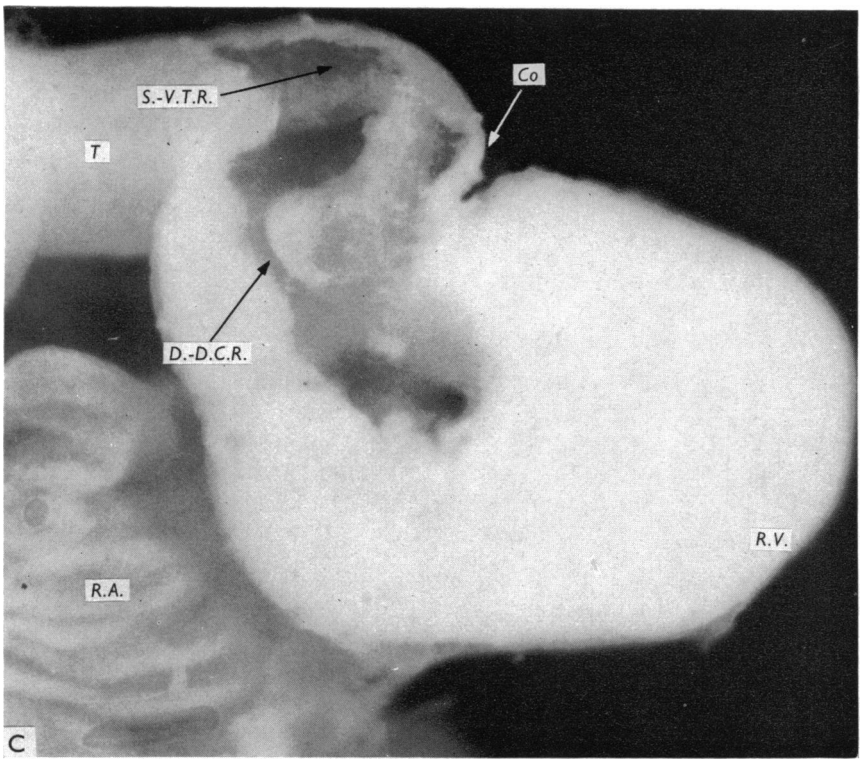

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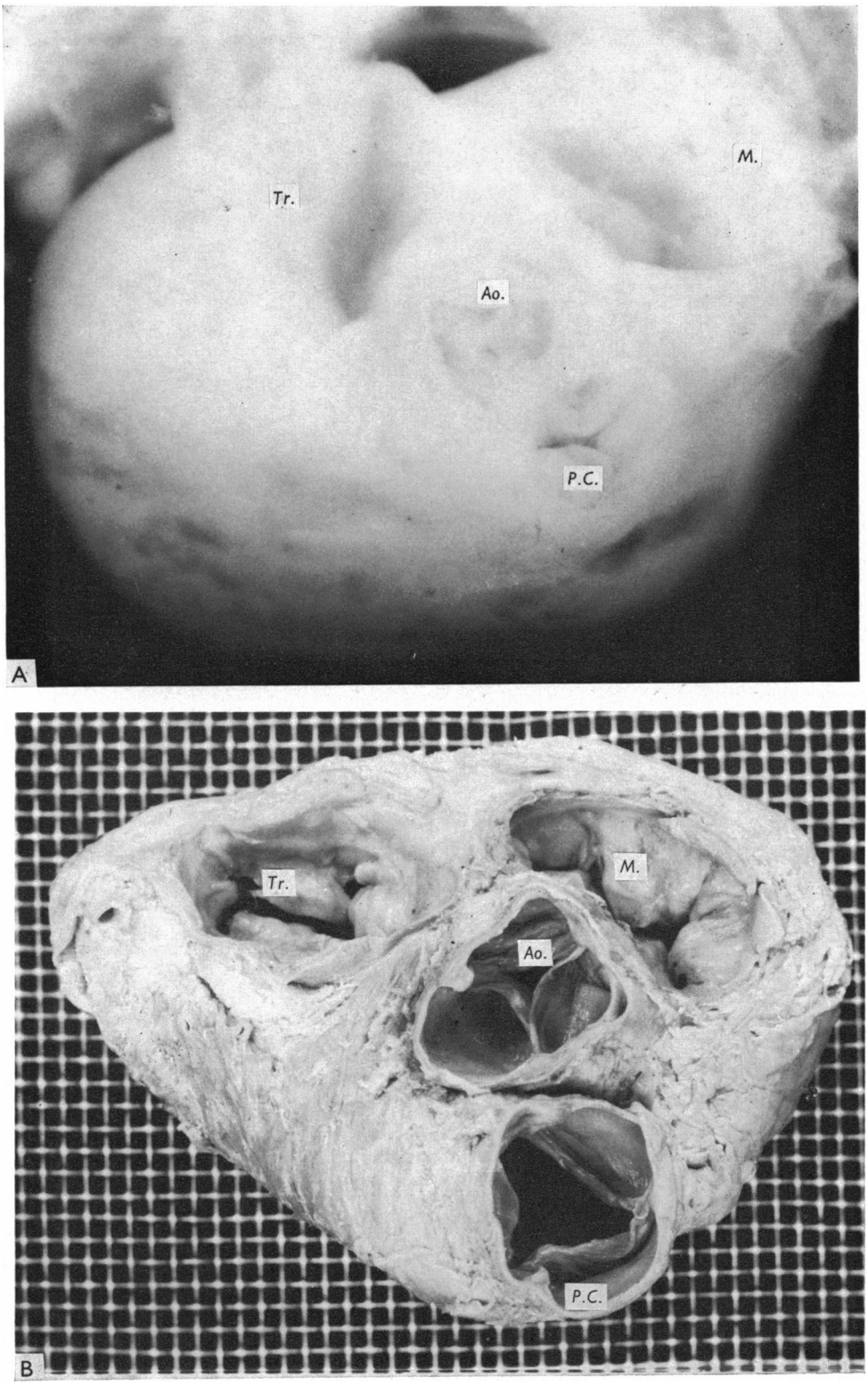
(label *d*) showed the label at stage 22 in the middle zone of the conus region or primordium of the infundibulum (compare Figs. 1B and 3A with Figs. 1C and 3B).

Series 3. In the 30 embryos labelled at stage 22, the labels were found as follows at stage 35. Label (*c*) was related to the inferior edge of the horizontal portion of the crista supraventricularis, which is the lower limit of the infundibulum or conus of the right ventricle. Label (*e*) was found in the annulus of the pulmonary semilunar valve cusps, which corresponds to the boundary between the conus and the truncus (compare Figs. 1C and 3C with Figs. 1D and 3D).

Fig. 4. Microdissections and transverse sections of the heart at stage 22 showing the relation of the conuses to one another, to the right ventricle, to the atrioventricular canal; and also the presence of the conus ridges. (A) Tranverse section showing two conuses, an anterior and right conus (1) and a posterior and left conus (2). Both become continuous with the trabeculated portion of the right ventricle (*R.V.*). ×63. (B) The right wall of the conus (*Co*) and of the right ventricle have been removed. Observe the conus ridges. Notice also that the posterior wall of the posterior conus (*P.W.P.C.*) forms part of the perimeter of the primary interventricular foramen (*P.I.-V.F.*). ×76. (C) The right wall of the conus and part of the wall of the truncus (*T*) have been removed. Notice the dextrodorsal conus ridge (*D.-D.C.R.*) which is continuous with the sinistroventral truncus ridge (*S.V.T.R.*). *R.A.*, right atrium; *L.A.*, left atrium; *A.-V.C.*, atrioventricular canal; *L.V.*, left ventricle; *S.-V.C.R.*, sinistroventral conus ridge. ×76.



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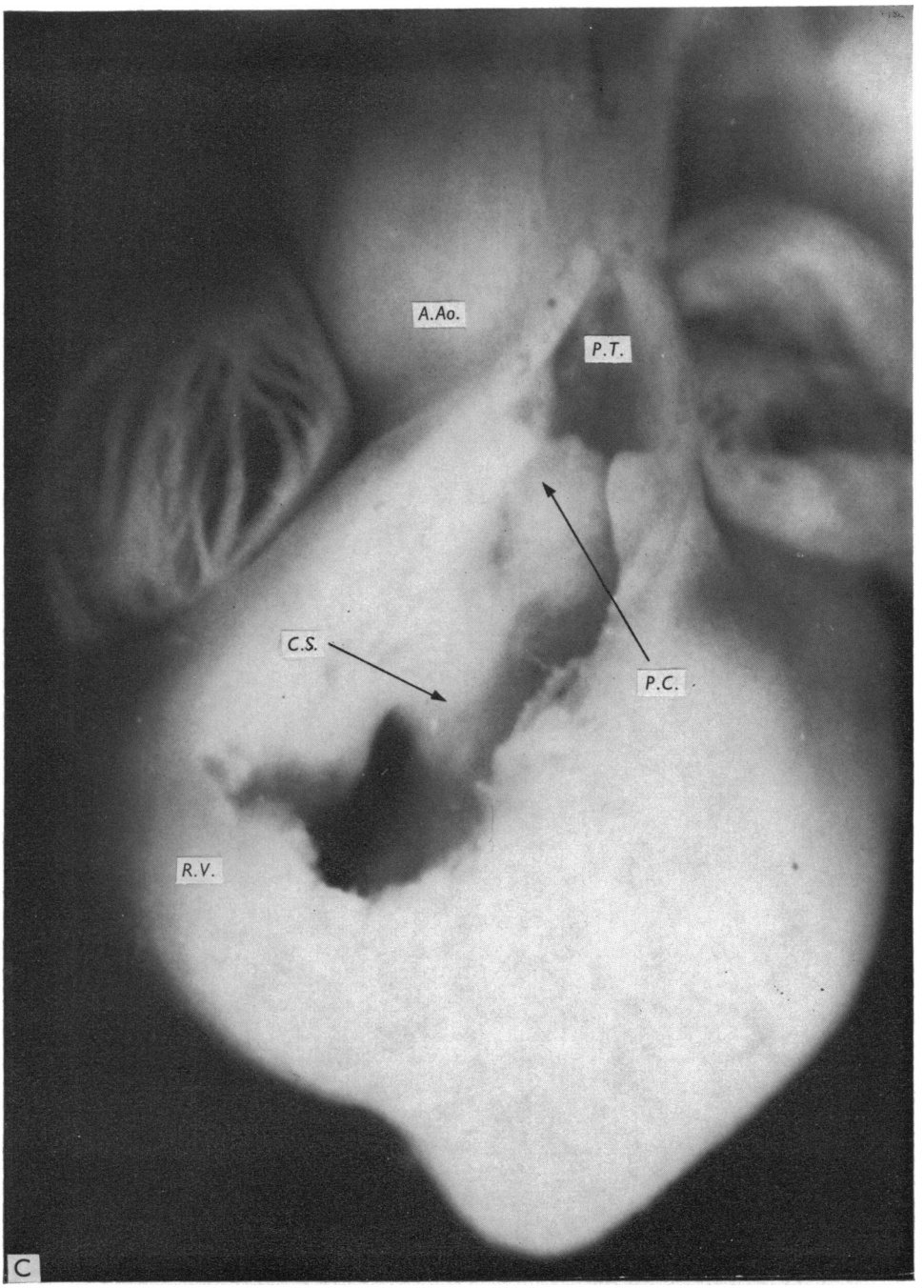

Fig. 5. Comparison of the mature heart of the chick embryo at stage 35 with the adult human heart. (A) Transverse section of the chick heart at the level of the valvular floors. × 33. (B) A similar section in the human heart. Notice that in the chick and in man the relations of the pulmonary and the aortic (*Ao.*) valvular floors to one another and of both to the mitral (*M*) and tricuspid (*Tr.*) orifices are similar. (C) Dissection of the infundibulum of the right ventricle (*R.V.*) and of the pulmonary artery trunk (*P.T.*) in the chick heart. × 34. (D) A similar dissection in the human heart. Observe that both in the chick and in man there is a great similarity in the position of the horizontal portion of the crista supraventricularis (*C.S.*), in the relation of the semilunar pulmonary valve cusps (*P.C.*) and in the relation of the trunk of the pulmonary artery with the ascending portion of the aortic arch (*A.Ao.*).

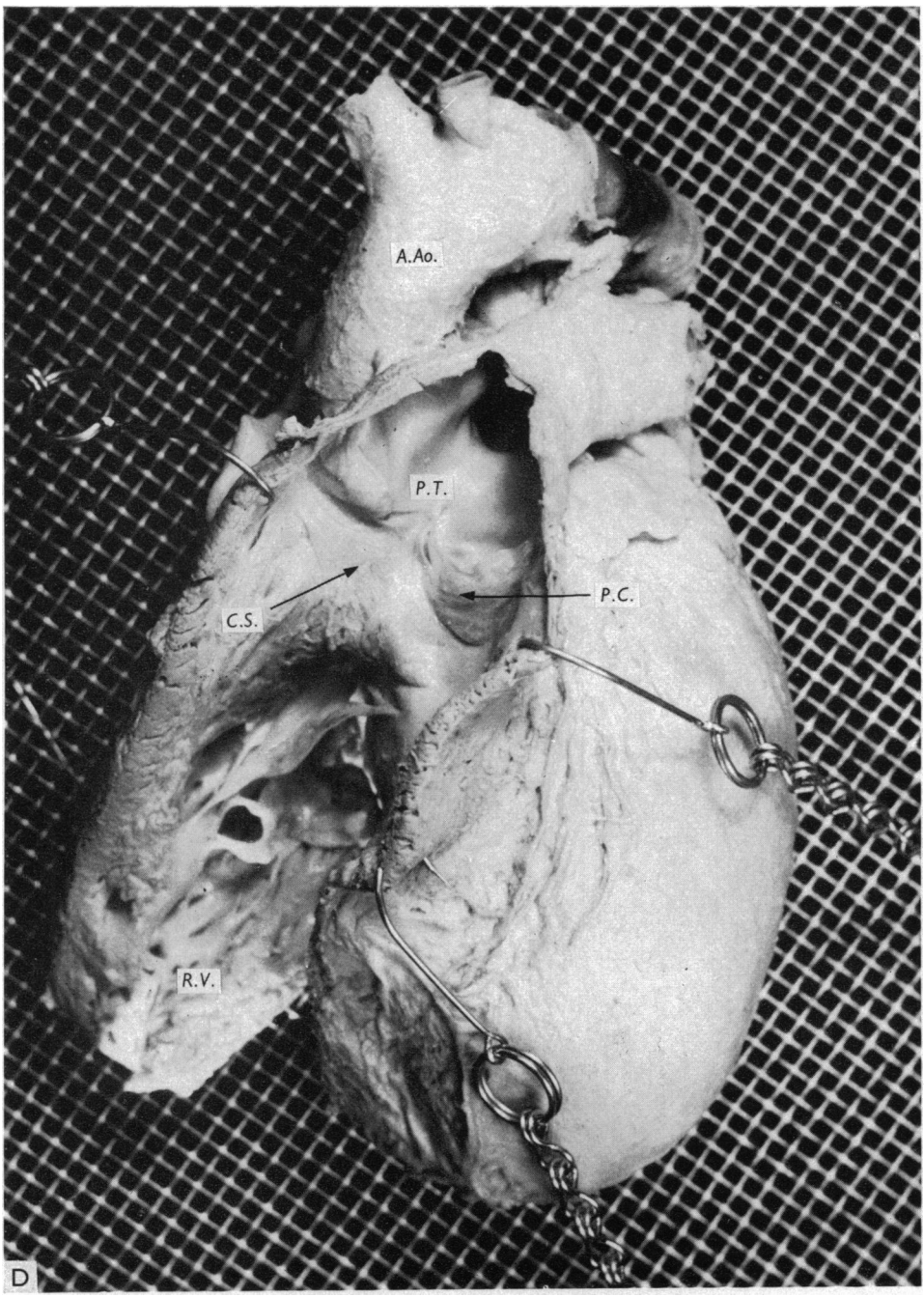

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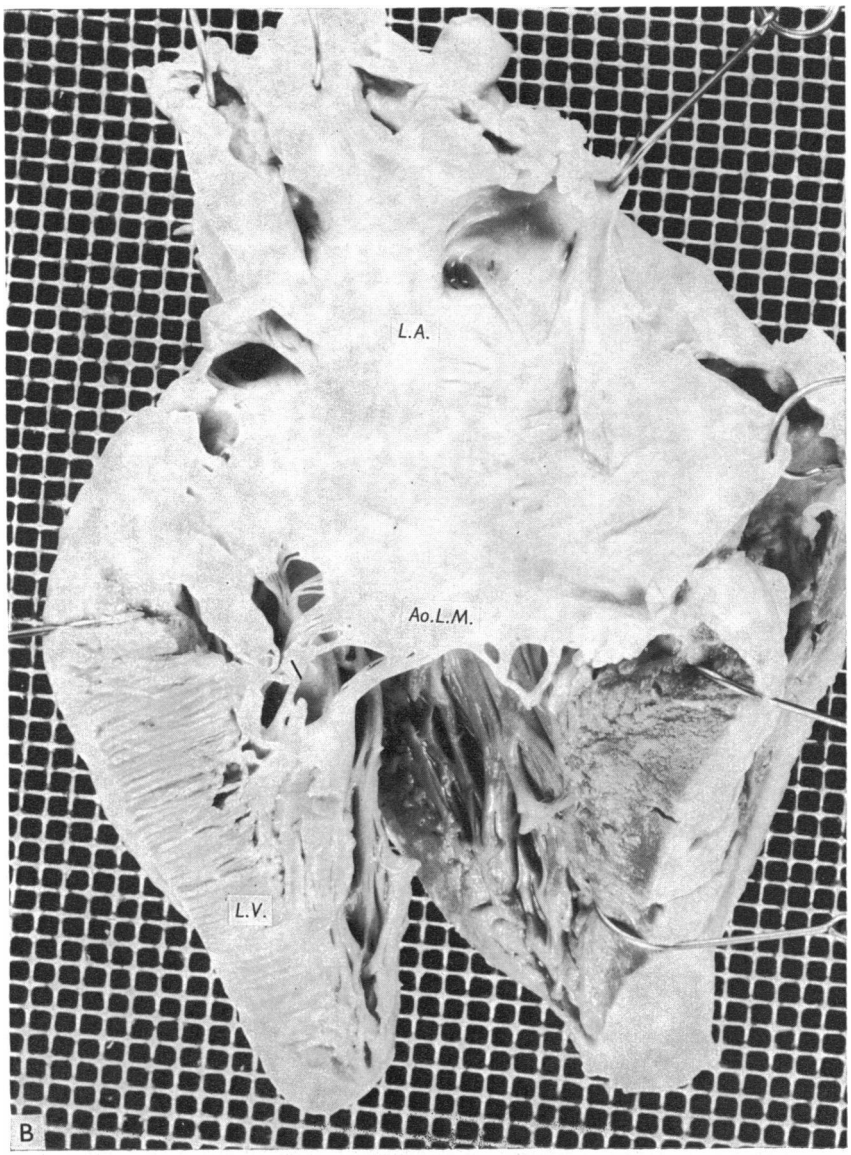
Fig. 6. Comparative study of the inflow and outflow chambers of the left ventricle in the mature heart of the chick embryo (stage 35) and in the adult human heart. (A) Dissection of the inflow chamber of the left ventricle (*L.V.*) of the chick heart. ×36. (B) A similar dissection in the human heart. Observe the similarity of both specimens as concerns the position of the aortic leaflet of the mitral valve (*Ao.L.M.*). (C) Dissection of the outflow chamber of the left ventricle in the chick heart. The aortic leaflet of the mitral valve was removed in order to show that at this stage of development the interventricular foramen is occluded, which indicates that the development of the conus has been completed. Notice the presence of the aortic semilunar valve cusps (*Ao.*). ×36. (D) Dissection of the outflow chamber of the left ventricle in man after removal of the aortic leaflet of the mitral valve. Observe the similarity of this area in the human and the chick heart. *L.A.*, left atrium.



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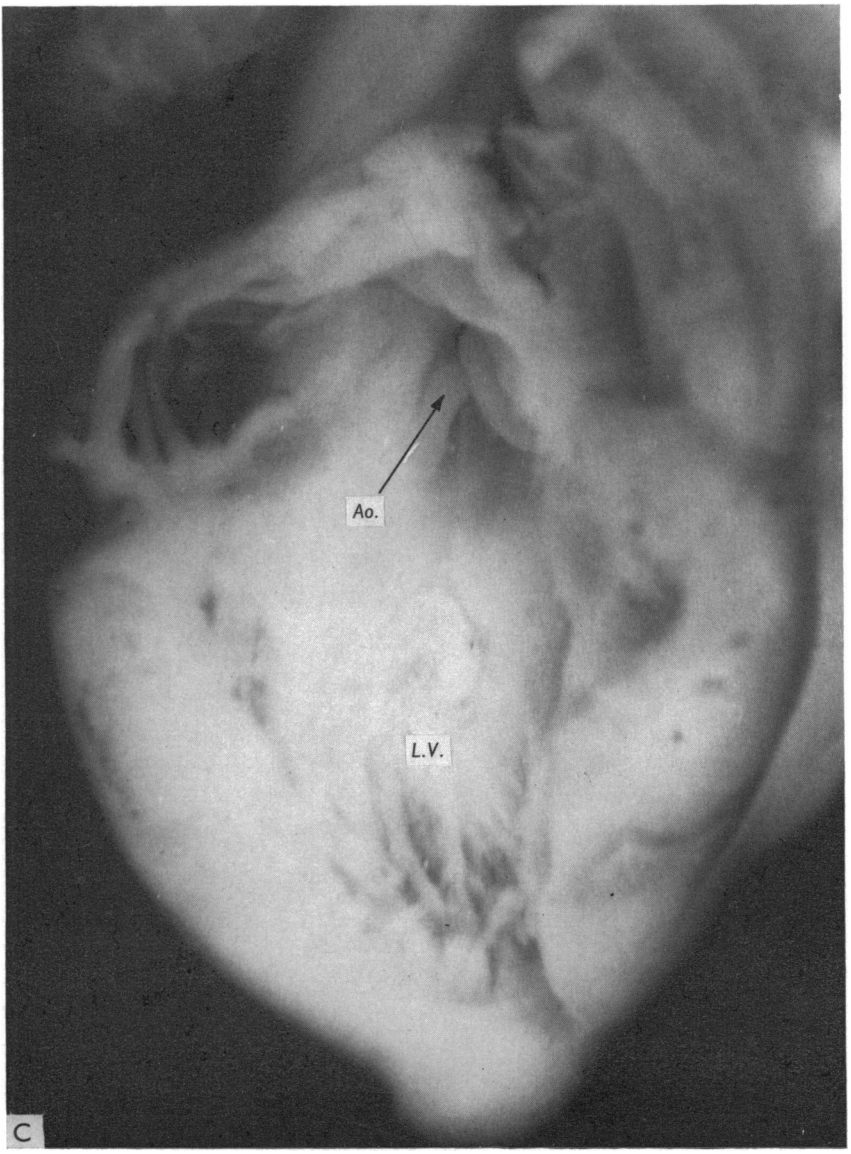
Anatomical microscopic studies

The transverse sections of the conus at the level of the atrioventricular canal (stage 22) showed that the longitudinal axis of the conus is caudocephalic while the longitudinal axis of the atrioventricular canal has a dorsoventral orientation (Fig. 4A). It was also shown at this same stage by means of placing small bits of hair in the lumen of the conus, that there are two conuses, one anterior and right, and the other posterior and left (Fig. 4A). Furthermore, the continuity of both conuses with the



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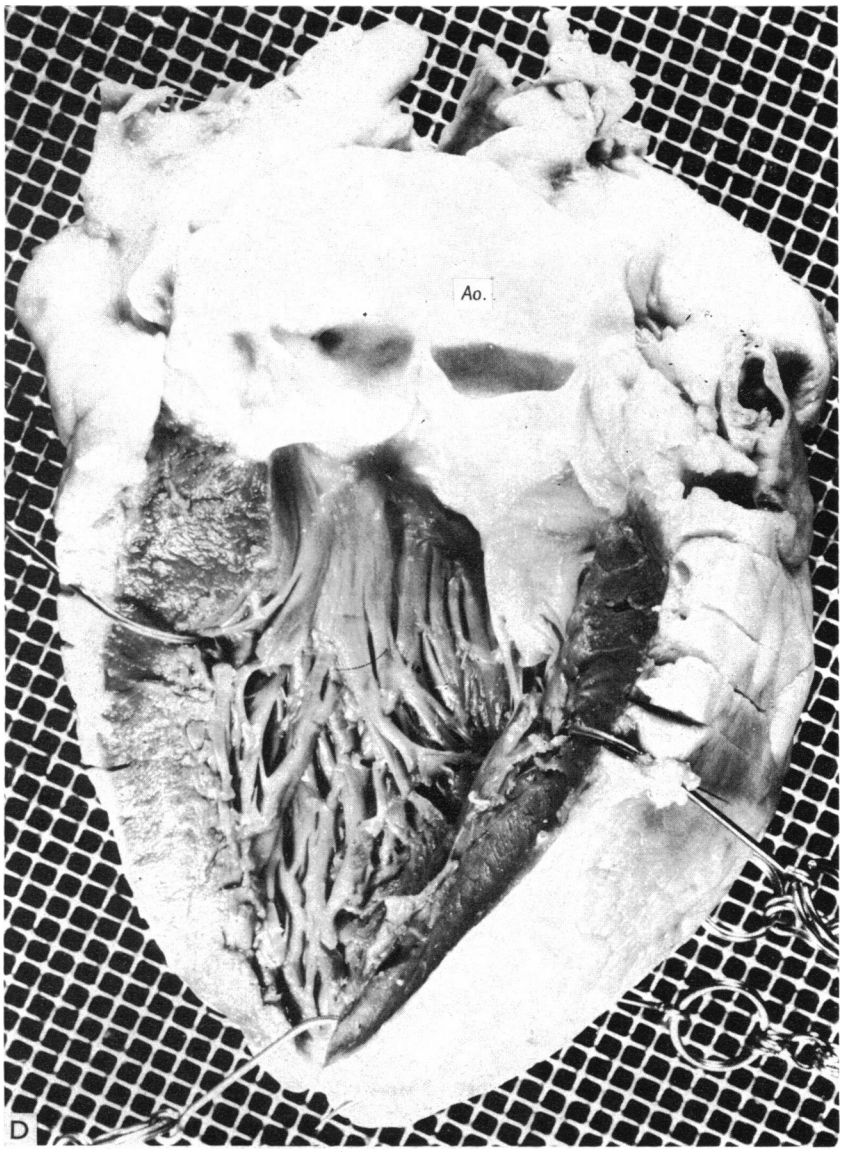
trabeculated portion of the right ventricle was made evident and also, the presence of a virtual space between the posterior wall of the posterior conus and the right surface of the atrioventricular canal (Figs. 4A, B). This shows that the left ventricle lacks an outflow chamber. Microdissections of the conus and the truncus from the right surface at this same stage proved that the two conus ridges are still not fused. In a plane posterior to the ridges, the posterior wall of the posterior conus becomes visible, which, together with the primitive interventricular septum, forms the peri-



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meter of the primary interventricular foramen (Figs. 4B, C). It was also proved that the dextrodorsal conus ridge is continuous with the sinistroventral truncus ridge and the sinistroventral conus ridge is continuous with the dextrodorsal truncus ridge, constituting two spiral structures (Fig. 4C).

Cinematographic studies done at stage 22 showed that the blood from the atria enters the left ventricle, which pumps it across the primary interventricular foramen into the right side chamber of the heart. This bloodstream is divided into two by

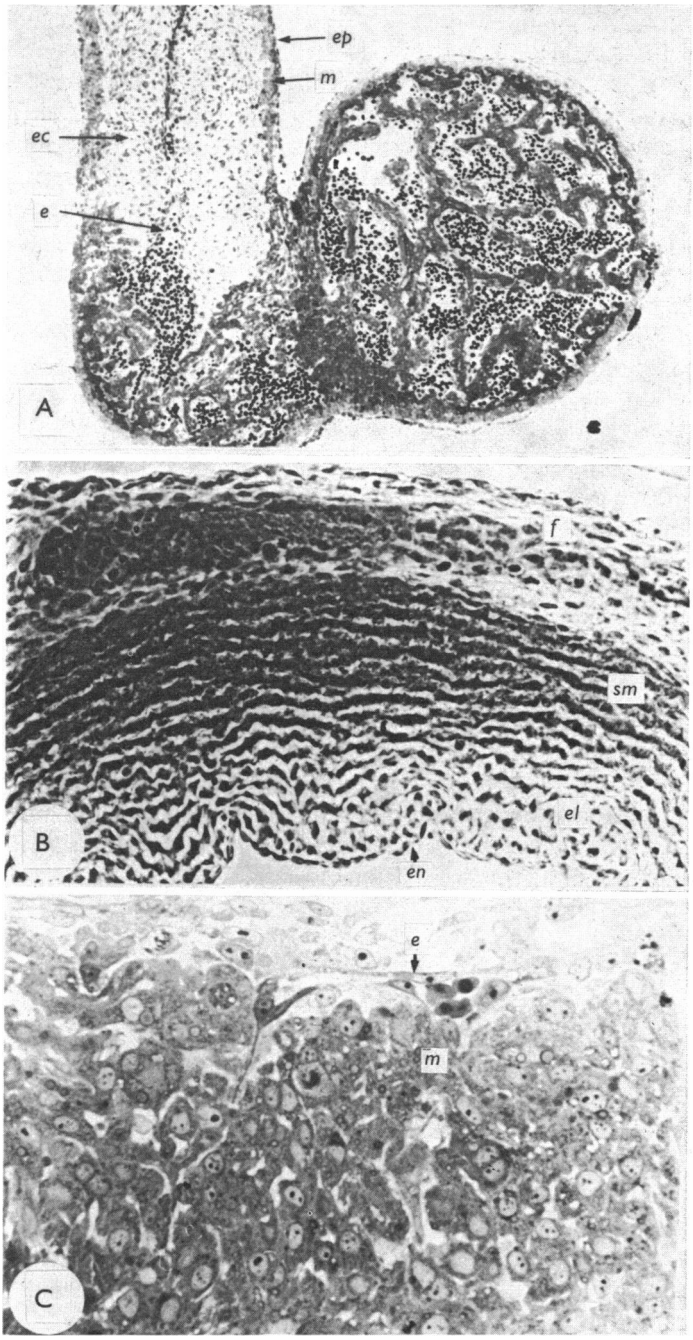

Fig. 7. Histology of the conus (stage 22), the pulmonary artery trunk, and the infundibulum of the right ventricle (stage 35). (A) Sagittal section of the embryonic chick heart at stage 22. This shows the conus formed externally by epicardium (*ep*) and myocardium (*m*) and internally by endocardium (*e*) and mesenchymal cells of the endocardial cushions (*ec*). × 120. (*B*) Transverse section of the pulmonary artery of the chick embryo at stage 35. Internally there is a fine layer of endothelium (*en*) overlying several layers of elastic fibres (*el*) and fibroblasts are visible. In the middle there are several layers of smooth muscle cells (*sm*), and externally there is a layer of connective tissue (*f*). × 200. (C) Transverse section of the infundibulum of the chick embryo at stage 35 showing its internal layers. This microphotograph shows a fine layer of endocardium (*e*) which lines the trabeculated wall of the myocardium (*m*). × 600.

the conus ridges; the greater one enters directly from the primary interventricular foramen into the posterior conus, while the smaller one enters the trabeculated portion of the right ventricle and from there is directed to the anterior conus. Both blood streams cross each other upon reaching the site where label (*e*) was placed, which is the boundary between the conus and the truncus. They continue, divided by the presence of the truncus ridges, until they reach the aortic arches. In this manner each stream forms a spiral. A certain amount of reflux directed towards the conus at the boundary between this structure and the truncus, was also observed.

Microdissections done at stage 35 showed that the right ventricular infundibulum has reached complete development; the horizontal portion of the crista supraventricularis is visible and the pulmonary artery, with its semilunar valve cusps, is also visible (Figs. 3D and 5C). Dissection of the left ventricular cavity, after having removed the aortic leaflet of the mitral valve, showed the aortic semilunar valve cusps and the interventricular septum were completely developed, i.e. there was no vestige of the interventricular foramen (Figs. 6A, C).

Dissections done in the infundibulum of the right ventricle showed that, both in man and in the chick (stage 35), the infundibulum is constituted by the horizontal portion of the crista supraventricularis, and also by a portion of the interventricular septum and of the free wall of the right ventricle, both of which are located in front of the parietal and septal insertions of the crista (Figs. 5C, D). Dissection of the infundibulum of the chick left ventricle also revealed that it is similar to that of the human heart, since in both it is formed by the aortic leaflet of the mitral valve, and also by the area of the interventricular septum and a portion of the free wall of the left ventricle, both of which are situated in front of that leaflet (Fig. 6). Transverse sections showed that the spatial relations between the valvular floors of the pulmonary artery and of the aorta are the same in man and in the chick, and the same was true of the relation between the two atrioventricular orifices and also of these orifices with the valvular floors of the great arteries (Figs. 5A, B).

Histological studies

The walls of the truncus and the conus at stage 22 are made up of a muscular layer about 5 cells thick. Some of these cells show small bands of myofibrils in their cytoplasm. The conal ridges contain more free mesenchymal cells surrounded by cardiac jelly and fibrous material (Fig. 7A) than the truncus ridges. The internal wall, both of the conus and the truncus, is covered by a fine layer of endocardium.

The infundibulum of the right ventricle at stage 35 shows an outer layer of epicardial cells, a middle layer of dense and trabeculated myocardial cells, and an inner layer of endocardium intimately associated with the muscular layer and following its invaginations and evaginations (Fig. 7B). The trunk of the pulmonary artery at stage 35 is formed by an external layer of fibroblasts, collagen and extracellular material, a middle layer with numerous smooth muscle cells, elastic fibres and other components of connective tissue and an internal layer of elastic fibres continuous with those of the middle layer (Fig. 7C), covered by endothelial cells which protrude into the lumen of the vessel.

DISCUSSION

The results of our *in vivo* labelling experiments on the developing chick heart indicate that this procedure is a valuable one for determining the prospective fate of different regions of this organ. Label (*c*), which was placed in the ventromedial region of the subcephalic fold at stage 9⁻, was found at stage 35, in the inferior edge of the horizontal portion of the crista supraventricularis (Figs. 1, 2 and 3C, D), which is the inferior anatomical limit of the infundibulum of the right ventricle in the mature heart (Figs. 3D and 5D). This fact permits us to conclude that the area of the heart in which label (*c*) appeared at stages 12 and 22 corresponds to the caudal limit of the primordium of the infundibulum or conus (Figs. 1, 2B, D and 3C, D). This, in turn, permits us to conclude: (1) That the region of fusion of the myocardial troughs at stage 9⁻ is not a 'conoventricular part', as was pointed out by Stalsberg & DeHaan (1969) and Castro-Quezada *et al.* (1972). (2) That the primordium of the infundibulum or conus appears at the bulboventricular loop stage (stage 12) (Figs. 1, 2B, D and 3C, D). (3) That at this same stage, the right interbulbar groove of Davis (1927), which according to this author separates the aortic bulb (truncus) from the bulbus cordis (conus plus primordium of the trabeculated portion of the right ventricle), actually separates the conus from the primordium of the trabeculated portion of the right ventricle (Figs. 1B and 2B). (4) That the caudal limit of the primordium of the infundibulum or conus at stage 22 corresponds to that mentioned by De Vries & Saunders (1962) in their descriptive embryological study at a similar stage of development of the human heart.

Now that the inferior limit of the primordium of the infundibulum or conus has been established by means of label (*c*), the results obtained with label (*e*) can be discussed. The latter was placed in the heart at stage 22 and was found in the mature heart (stage 35) in the annulus of the pulmonary semilunar valve cusps (Figs. 1C, D and 3C, D), which is the upper limit of the infundibulum. This result enables us to make the following conclusions: (1) At stage 22, the area in which label (*e*) was placed will give origin to the semilunar valve cusps of the great arteries, as has been pointed out by other authors (De Vries & Saunders, 1962). (2) This region is the boundary between the primordium of the infundibulum or conus and the truncus, as has been suggested by De Vries & Saunders (1962) for human embryos which have a cardiac morphology similar to that of stage 22 of the chick embryo. If a correlation is made at stage 22 between the position of labels (*c*) and (*e*) we may conclude that the primordium of the infundibulum or conus at this stage corresponds to the region of the heart between the primordium of the truncus distally and the primordium of the trabeculated portion of the right ventricle proximally (compare Figs. 1C and 3C with 1D and 3D).

A study of label (*d*) placed in the cephalic end of the cardiac tube at stage 12 (loop stage), which was found at stage 22 in the middle area of the primordium of the infundibulum or conus (Figs. 1B, C and 3A, B), permit us to conclude that the segment of the heart between labels (*c*) and (*d*) at stage 12 is a portion of the primordium of the infundibulum or conus (compare Figs. 1B, 2C and 3A with 1C and 3B, C) and that it constitutes the cephalic end of the cardiac tube. Therefore,

the primordium of the truncus probably appears between stages 13 and 22, since stage 12 only shows the caudal portion of the primordium of the infundibulum or conus (Figs. 1B, C).

It is of interest to discuss results obtained with the labelling technique of areas between (*a*) and (*b*), and (*b*) and (*c*) at stage 9⁻ which are found at stage 12 in the primordium of the trabeculated portion of the right ventricle (Figs. 1A, B and 2A, B). In this way, the portion between (*a*) and (*b*), which is the region of fusion of the myocardial troughs, is not the primordium of the conus (Castro-Quezada *et al.* 1972; Stalsberg & DeHaan, 1969), but rather the caudal region of the primordium of the trabeculated portion of the right ventricle. At the same time, the one between (*b*) and (*c*), described as cardiogenic (Argüello *et al.* 1975), corresponds to the cephalic region of the primordium of the trabeculated portion of the right ventricle (Figs. 1A, B and 2A, B).

Consideration of the primordia of the truncus and the conus with respect to their location and time of appearance during development, as done in this study by means of *in vivo* labelling, as well as research done by Stalsberg & DeHaan (1969) and by Castro-Quezada *et al.* (1972) with respect to the prospective interventricular groove (formerly considered as atrioventricular groove), permits us to conclude that the primitive cardiac cavities appear in different developmental stages of the heart and, therefore, not all the cavities are present in the pre-loop stage, as has been established by other researchers (Davis, 1927; De Vries & Saunders, 1962; Grant, 1962; Van Mierop & Netter, 1969; De la Cruz *et al.* 1971; Goor *et al.* 1972; Anderson *et al.* 1974).

Once the precise limits of the primordium of the infundibulum or conus at stage 22 had been established by means of the labelling techniques, it was possible to undertake a careful study of the anatomy of this region (Compare C with D in Fig. 3). We confirmed what De Vries & Saunders (1962), and Van Mierop & Netter (1969) had established, i.e. the existence of an anterior right conus and a posterior left conus, separated by the conal ridges which are still not fused (Figs. 4A, B), as had been pointed out by De Vries & Saunders (1962) for the human embryo heart. We also verified that both conuses are continuous with the trabeculated portion of the right ventricle by their caudal end (De Vries & Saunders, 1962), without an anatomical continuity between the posterior left conus with the left ventricle (Fig. 4A), as has also been mentioned by Van Mierop *et al.* (1962) and by Van Mierop & Netter (1969). Furthermore, we observed that the posterior wall of the posterior conus is apposed to the right wall of the atrioventricular canal, whose longitudinal axis is dorsoventral, while that of the primordium of the conus is cephalocaudal (Fig. 4A). Another interesting observation is that which confirms statements by other authors (Kramer, 1942; De Vries & Saunders, 1962; Van Mierop *et al.* 1963; Van Mierop & Netter, 1969) with respect to the truncoconal ridges, viz. that the sinistroventral conus ridge is continuous with the dextrodorsal truncus ridge, while the dextrodorsal conus ridge is continuous with the sinistroventral truncus ridge (Fig. 4C), and both cross each other at the level of the truncoconal junction. This fact is made visually evident by cinematography.

The anatomical results obtained by means of dissection of the mature chick heart (stage 35) and of man, permit us to indicate that both are similar as concerns the

infundibulum of the right ventricle and of the left ventricle, the position of the trunk of the pulmonary artery with respect to the ascending portion of the aortic arch, the relations of the valvular floors of the great arteries and the relations of these with the atrioventricular orifices (Figs. 5 and 6). Furthermore, the presence of similar morphologies during the development of the truncus and of the conus in the chick and in man permits us to conclude that the information obtained by means of *in vivo* labelling techniques in the chick embryo are indispensable in evaluating the results obtained from the study of the truncus and the conus in the human embryo by means of descriptive embryological techniques, since it is obvious that experimental techniques are not applicable to the human embryo.

Histological studies of the primordia of the conus and of the truncus at stage 22 show that the walls of both are constituted by cardiac muscle. However, at stage 35, the walls of the trunk of the pulmonary artery acquire the histological features of the walls of great arteries, while the infundibulum of the right ventricle maintains the features pertaining to cardiac muscle (Fig. 7). Prospective research under way indicates that the transformation of the cardiac muscular wall of the truncus into connective tissue and smooth muscle begins at stage 28 (Argüello & De la Cruz, 1975).

SUMMARY

The development of the truncus and the conus was studied in the chick embryo by *in vivo* labelling techniques. The earliest labels were placed at the stage of fusion of the myocardial troughs (stage 9$^-$) and they were traced until the mature heart stage (stage 35). Microdissections and light microscopic studies were also carried out. The results are discussed in relation to the human heart.

Our experiments permit the following conclusions: (1) At stage 9$^-$ fusion of the myocardial troughs takes place at the level of the primordium of the trabeculated portion of the right ventricle, when neither the conus nor the truncus are present. (2) At stage 12 (loop stage) there appears the caudal portion of the conus, which constitutes the cephalic end of the cardiac tube. (3) The truncus appears between stages 13 and 22. (4) At stage 22 angular junction between the conus and the truncus is the area where the semilunar valve cusps of the great arteries will develop and that, at this same stage, the junction between the conus and the trabeculated portion of the right ventricle seen from the right surface corresponds to the inferior edge of the crista supraventricularis. (5) It was confirmed that the pulmonary semilunar valve cusps originate from the walls of the truncus. (6) The development of the conus and truncus are similar in chick and man. (7) Histologically, in the chick, the wall of the truncus and the conus contain cardiac muscle as late as stage 28, but from then on the walls of the truncus are transformed into connective tissue and plain muscle.

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